# Synapse structure: **Glutamate receptors connected by the shanks**

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**A family of proteins has been identified whose members, the Shanks, physically link two major receptor complexes at excitatory synapses – NMDA receptors and metabotropic glutamate receptors.**

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Communication between neurons depends on a highly ordered and regulated arrangement of proteins at synapses. Such organization is typified by the selective localization of neurotransmitter receptors and signaling molecules opposite appropriate presynaptic terminals. At excitatory synapses in the brain, receptors for the neurotransmitter glutamate are clustered in the postsynaptic membrane, where they play crucial roles in synaptic transmission and synaptic plasticity. Because of their importance in brain function and dysfunction, the molecules and mechanisms involved in localizing glutamate receptors and associated signaling proteins to excitatory synapses have been the objects of intense scrutiny.

The excitatory neurotransmitter glutamate acts on two principal classes of postsynaptic receptors: ionotropic glutamate receptors and group I metabotropic glutamate receptors. Ionotropic glutamate receptors are glutamate-gated ion channels responsible for rapid synaptic transmission and ion flux across the postsynaptic membrane. Metabotropic glutamate receptors are G-protein-coupled receptors that influence neuronal excitability, synaptic transmission and synaptic plasticity through the activation of second messenger cascades. Both types of glutamate receptor are concentrated together at the postsynaptic membrane of excitatory synapses.

The colocalization of these structurally and functionally diverse receptors suggests a common downstream organizing mechanism. A wealth of studies in the last few years have nevertheless found that ionotropic glutamate receptors and metabotropic glutamate receptors interact with distinct anchoring/scaffolding proteins through their carboxy-terminal cytoplasmic domains [1]. Now, however, studies in the laboratories of Morgan Sheng and Paul Worley [2,3] have identified a novel family of scaffold proteins, known as the Shanks, which may bridge the gap between the ionotropic and the metabotropic glutamate receptors.

In the first of the new papers, Naisbitt *et al.* [2] focused on the protein complex associated with the *N*-methyl-D-aspartate-type glutamate receptors (NMDA receptors), a subtype of ionotropic glutamate receptors that is particularly important for synapse formation and synaptic plasticity. At the core of this complex is PSD-95, the prototypical member of a family of proteins involved in clustering ion channels [1]. PSD-95 consists of little more than a series of protein interaction motifs, including three PDZ domains, an SH3 domain and a guanylate kinase (GK) homology domain. PSD-95 interacts with NMDA receptors through either of its first two PDZ domains, leaving its other protein-binding domains available to interact with additional synaptic proteins.

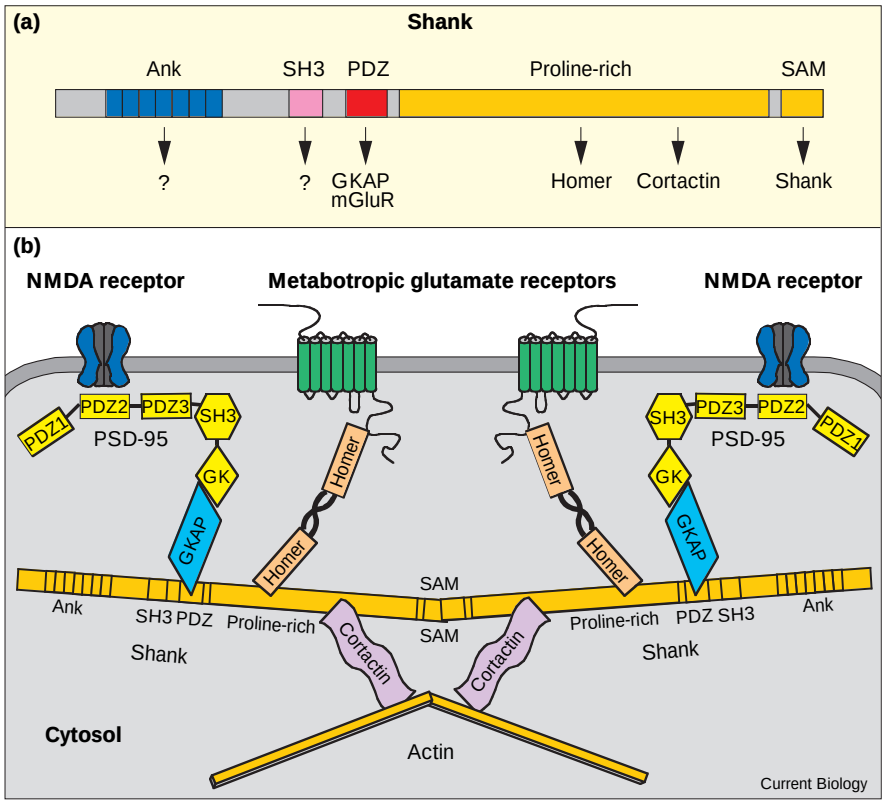
One such group of proteins is the family of 'GK-associated proteins' (GKAPs), which are enriched in the postsynaptic density where, as their names suggest, they associate tightly with the GK domain of PSD-95 [4]. The amino-acid sequences of the GKAPs have revealed little about their function. One conspicuous feature at the carboxyl terminus of the GKAPs, however, is the tetrapeptide QTRL, which is reminiscent of sequences that bind to PDZ domains [1]. With this in mind, Naisbitt *et al.* [2] sought to address the function of GKAPs and elaborate on the NMDA receptor–PSD-95–GKAP complex by identifying further GKAP-interacting proteins.

Using the carboxy-terminal region of GKAP as bait in a yeast two-hybrid screen, Naisbitt *et al.* [2] identified the Shank protein family. The Shank proteins are products of at least three related genes, *Shank1–Shank3*, and a number of different transcripts; below they are collectively referred to as Shank. Shank possesses multiple protein interaction motifs, but no apparent catalytic domains. These interaction motifs include seven amino-terminal ankyrin repeats, an SH3 domain, a PDZ domain, a long proline-rich segment and a carboxy-terminal SAM domain (Figure 1a). As expected from its carboxy-terminal QTRL sequence, GKAP interacts with the PDZ domain of Shank. Thus, as with PSD-95, the domain organization of Shank implies that it functions as a multivalent scaffolding protein.

Several lines of evidence support the notion that Shank interacts with the NMDA receptor–PSD-95–GKAP complex at excitatory synapses [2]. First, Shank, GKAP and PSD-95 were found to co-cluster in heterologous cells. Furthermore, Shank coimmunoprecipitated with PSD-95/GKAP from rat brain extracts, and was seen to colocalize with NMDA receptors at excitatory synapses. Moreover, biochemical fractionation and immunogold

**Figure 1**

(a) Domain organization and protein binding partners of Shank. (b) A schematic diagram of the postsynaptic membrane of an excitatory synapse, illustrating the proposed linkage of Shank to NMDA receptors, metabotropic glutamate receptors and the actin cytoskeleton. (See text for details.)




electron microscopy both placed Shank in the postsynaptic density along with NMDA receptors, PSD-95 and GKAP. Perhaps most convincingly, expression of GKAP splice variants that lack the QTRL Shank-binding domain in cultured neurons reduced the number of synaptic Shank clusters, indicating that efficient localization of Shank to synapses requires interaction with GKAP. These findings place Shank downstream of PSD-95 and GKAP in a linear progression of protein–protein interactions, three steps removed from the NMDA receptor (Figure 1).

Meanwhile, in an effort to trace protein interactions back from metabotropic glutamate receptors, Tu *et al.* [3] started with Homer. The Homer proteins are a family of cytosolic adaptor proteins found at excitatory synapses that interact with group I metabotropic glutamate receptors and functionally link them to inositol trisphosphate ($IP_3$) receptors, the major sites of intracellular $Ca^{2+}$ release [5,6]. Most Homer proteins have an amino-terminal EVH1 domain, which binds proline-rich motifs in metabotropic glutamate receptors and $IP_3$ receptors, and a carboxy-terminal coiled-coil domain, which mediates their oligomerization [5,6]. To identify additional binding partners of the Homer EVH1 domain, Tu *et al.* [3] also turned to the yeast two-hybrid system, and in so doing also isolated Shank [3]. They found that Shank interacts with Homer proteins both in heterologous cells and *in vivo*. The Homer-binding domain of Shank mapped to a conserved sequence within its proline-rich segment [3], thereby distinguishing the Shank–Homer interaction from the PDZ-mediated Shank–GKAP interaction [2] (Figure 1).

Are Shank proteins involved in clustering both NMDA receptors and metabotropic glutamate receptors? A role for Shank in clustering and anchoring metabotropic glutamate receptors is supported by the observation that, by immunogold electron microscopy, Shank and Homer appear to colocalize at the postsynaptic density, at sites that are also enriched for metabotropic glutamate receptors [3]. Furthermore, Shank–Homer complexes cluster metabotropic glutamate receptors in heterologous cells, although neither protein does so alone. This is remarkable given that Shank can also interact directly with the cytoplasmic tails of metabotropic glutamate receptors through its PDZ domain [3]. Perhaps most provocative is the finding that Homer and PSD-95 co-cluster when coexpressed with GKAP and Shank in heterologous cells [3]. These studies thus suggest that Shank proteins form a molecular bridge indirectly linking the NMDA receptor complex, through PSD-95–GKAP, with metabotropic glutamate receptors or $IP_3$ receptors, through Homer (Figure 1b).

The ability of Shank proteins to interact with both the NMDA receptor–PSD-95–GKAP complex and the metabotropic glutamate receptor–Homer complex indicates that they may act as central organizers of the postsynaptic density. Other features of Shank proteins add further support for this view. For example, they are able to oligomerize with one another via their SAM domain [2], a domain known in other contexts to mediate protein oligomerization [7]. The formation of extended polymeric arrays of Shank proteins might be important for crosslinking multiple groups of NMDA receptor–PSD-95–GKAP or metabotropic glutamate receptor–Homer complexes in the postsynaptic density.

Shank proteins may also couple to the actin cytoskeleton via cortactin, an F-actin-binding protein implicated in actin remodeling at the cell periphery [8]. Indeed, Shank proteins share considerable sequence homology to the cortactin-binding protein CortBP1 [9], which is also enriched at synapses [10]. Cortactin can bind a sequence within the proline-rich segment of Shank distinct from the Homer-binding domain (Figure 1a) [2]. Interestingly, although there is very little colocalization of Shank with cortactin in mature neurons, the extent of colocalization increases after stimulation with glutamate [2]. This could conceivably reflect an activity-dependent interaction of cortactin with Shank that is important for actin remodeling at the synapse. The ability of Shank to self-associate and bind cortactin underscores the multiplicity of its potential functions and lends credence to its proposed role as a higher-order scaffolding protein.

The identification of the Shank family of scaffolding proteins provides new insights into the molecular structure and organization of excitatory synapses. These new studies define an array of synaptic protein interactions emanating from Shank which may link NMDA receptors and metabotropic glutamate receptors to each other, as well as to the actin cytoskeleton and $IP_3$ receptor $Ca^{2+}$ release sites. In this context, Shank is well-positioned to contribute to the assembly of the postsynaptic density during synaptogenesis and to promote crosstalk between glutamate receptor signaling pathways. Undoubtedly, the identification of the Shank family will pave the way for a more detailed molecular dissection of the postsynaptic density. We can almost certainly look forward to the discovery of new Shank binding partners, which may ultimately provide links to other receptor complexes, such as the AMPA-type ionotropic glutamate receptors. In addition, more detailed functional studies of Shank proteins should illuminate key features of synaptogenesis and synaptic plasticity. In this regard, Shank promises to provide a major leap in our understanding of synapse structure and function.

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